

Comments of the Chlorine Chemistry Council  
on the National Toxicology Program's Classification of 2,3,7,8-TCDD  
in the Ninth Report on Carcinogens

**Introduction**

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* (The Report) lists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as *Reasonably Anticipated to be a Human Carcinogen*. For the ninth edition of this report, NTP is proposing to change the listing of TCDD to the *Known to be a Human Carcinogen* category. The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, believes that the science does not support this proposed change, and urges the NTP Board of Scientific Counselors to advise NTP not to list TCDD as a known human carcinogen. CCC submits the following brief comments concerning the proposed TCDD listing. CCC submitted more extensive comments to NTP in August 1997 (CCC's August Comments).

NTP's recently revised criteria for listing chemicals as known human carcinogens requires "sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent . . . and human cancer" (61 Fed. Reg. 50,499 (September 26, 1996)). As demonstrated below and discussed more fully in CCC's August Comments, the epidemiology data for TCDD do not establish a causal relationship between TCDD exposure and cancer. The studies reporting an association between TCDD exposure and human cancers suffer from confounding, inconsistency, lack of specificity, and weak associations. Indeed, the U.S. Environmental Protection Agency (EPA), EPA's Science Advisory Board (SAB) and an International Agency for Research on Cancer (IARC) Epidemiology Working Group have concluded that the epidemiological data for TCDD are only "limited" or "suggestive." Therefore, TCDD epidemiology data do not support a change in NTP's current carcinogen listing for TCDD.

The recently revised NTP criteria also state that data related to a substance's mechanism of action may be considered. Beyond binding with the Ah receptor, the mechanism of carcinogenic

action for TCDD is unknown. Therefore, there is no mechanistic basis for assuming that TCDD is carcinogenic in humans simply because it has been found to be carcinogenic in some animal studies. In fact, the dramatic differences between the half lives of TCDD in animals and humans (e.g., 10 days versus 10 years) may demonstrate a significant biochemical difference between animals and humans.

NTP's criteria are clear: when there is evidence of carcinogenicity in animals, limited evidence in humans, and insufficient mechanistic data, a substance is to be classified as *Reasonably Anticipated to be a Human Carcinogen*.

### **The Epidemiological Data for TCDD Are Not Sufficient to Support Listing TCDD as a Known Human Carcinogen**

Unlike NTP's current list of known human carcinogens, the weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria (e.g., consistent findings, a strong association, and evidence of a dose-response relationship). Indeed, EPA, the SAB and IARC have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive."

The numerous epidemiological studies of human populations (even those exposed to unusually high levels of TCDD) do not demonstrate that TCDD is a known human carcinogen. EPA, after a lengthy and careful analysis (which included extensive input from the scientific community), decided not to classify TCDD as a Group A carcinogen. The SAB supported this decision, stating "...virtually all of the Committee believes that the studies of humans [would be categorized] as 'limited' providing for an overall evaluation...as 'Probably Carcinogenic to Humans' with limited supporting information from human studies" (*An SAB Report: A Second Look at Dioxin*, p. 67).

EPA cites four key epidemiological studies in its *Draft Dioxin Reassessment* as the best evidence demonstrating the carcinogenicity of TCDD (Fingerhut et al. 1991, Saracci et al. 1991, Zober et al. 1990, and Manz et al. 1991). All involve workers occupationally exposed, many of whom had chloracne (indicating very high exposures). The Fingerhut study reported an excess of respiratory cancers and all cancers combined. The authors concluded that the elevated risk for

"all cancers combined" was consistent with the carcinogenic effects of TCDD observed in animal studies. However, not a single tumor observed in the animal studies was found, nor were cancers predicted from previous epidemiological studies observed. Likewise, the studies by Saracci et al., Zober et al., and Manz et al. essentially failed to report "cancers of a priori interest." In reviewing these data, EPA concluded that "[t]he epidemiology evidence for a TCDD lung cancer hazard in humans is suggestive, but not conclusive, while that for all cancers combined has less certainty" (*EPA Draft Dioxin Reassessment Revised Chapter 8, Dose-Response Modeling*, p. 121). These studies, including EPA's and the SAB's findings concerning these studies, are discussed in more detail in CCC's August Comments.

The RC Background Document for TCDD (RC Document) claims, "the strongest epidemiological evidence for the carcinogenicity of 2,3,7,8-TCDD is for all cancers combined, rather than for any specific site" (Appendix A, p. 337). Yet even for this finding, the relative risk of 1.4 does not constitute compelling evidence, particularly when confounding cannot be excluded. Furthermore, known human carcinogens (e.g., smoking, ionizing radiation) which produce tumors at more than one site also demonstrate unequivocal elevated risks for specific sites. In fact, there is no known compound that is a multi-site carcinogen without particular sites predominating (*Id.*). This strongly suggests that the epidemiological evidence for TCDD is more consistent with exposures to multiple chemicals rather than exposure to a single chemical, such as TCDD.

A number of studies have been published since the release of the Draft *Dioxin Reassessment* and SAB's critical review of that document. An IARC Epidemiology Working Group conducted a thorough review of the epidemiological data, including the NIOSH study from the United States (Fingerhut et al. 1991), studies from the Netherlands (Bueno de Mesquita et al. 1993) and Germany (Ott and Zober 1996 and Becher et al. 1996), an international study by IARC in which these and other data were aggregated (Kogevinas et al. 1996), and the most recent study of the Seveso cohort (Bertazzi et al. 1996). The Working Group found that even these additional studies were not sufficient to alter the conclusion that the epidemiological data were "limited" and failed to demonstrate a causal relationship between exposure to TCDD and human cancer. These studies, and their limitations are also discussed in CCC's August Comments.

Current Information Concerning the Mechanism of Action for TCDD-Induced Carcinogenicity Does Not Support Changing the TCDD Carcinogenicity Listing

NTP's recently revised listing criteria state that mechanism of action may also be considered when drawing conclusions regarding carcinogenicity in humans or experimental animals.<sup>1</sup> Presumably, if mechanistic data were used to move a substance from the *Reasonably Anticipated to be a Human Carcinogen* list to the *Known to be a Human Carcinogen* list, NTP must demonstrate that the mechanism in animals is at least qualitatively similar in both animals and humans. This would require a detailed understanding of the mechanism of action for a specific substance in both experimental animals and humans.

In the case of TCDD, little is known about its mechanism of action in animals, and even less is known about its mechanism of action in humans. Therefore, current mechanistic data cannot support moving TCDD to the *Known to be a Human Carcinogen* list. As discussed below, the current hypotheses concerning possible TCDD tumor-inducing mechanisms are merely speculation. What is known and generally accepted at this time concerning TCDD mechanisms of actions is that TCDD, like a number of other substances, binds to the aryl hydrocarbon (Ah) receptor and results in the induction of certain enzymes. Neither of these events, however, are sufficient to explain how adverse responses, particularly cancer, occur. Apart from receptor binding and enzyme induction, little is known, much less understood, about mechanistic events leading to TCDD-induced carcinogenicity in animals. Nonetheless, the RC Background Document for TCDD (RC Document) relies on Ah receptor binding to incorrectly imply that animals and humans share the same mechanism of carcinogenic action for TCDD.

Not only is there insufficient information to conclude that animals and humans share the

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<sup>1</sup> It is not clear whether mechanistic data can be used when epidemiological data are insufficient to change the listing of a chemical from *Reasonably Anticipated to be a Human Carcinogen* to *Known to be a Human Carcinogen*. Indeed, the only example provided in the revised criteria concerns removing an animal carcinogen from listing when evidence demonstrates that the substance, for mechanistic reasons, is not anticipated to be carcinogenic in humans.

same mechanism of carcinogenic action for TCDD, some evidence suggests that animals and humans may exhibit important mechanistic differences after initial Ah binding. As discussed elsewhere in the RC Document, TCDD has a strikingly different half-life in rodents than in humans (10-30 days compared 5.8-11.3 years, respectively). This important distinction suggests that animals and humans do not share the same mechanism of carcinogenic action for TCDD because TCDD is metabolized differently, possibly through different enzyme systems or transcriptional activity. Further, if TCDD exerts a carcinogenic effect in animals through the activity of a metabolite, rodents may be much more sensitive to TCDD than humans.

A true mechanism of action for a chemical must be both necessary and sufficient with respect to explaining how the chemical induces toxicity in animals. This is clearly not the case for Ah receptor binding and enzyme induction for TCDD. EPA has stated that "[b]inding to the Ah receptor appears to be necessary for all well-studied effects of dioxin but is not sufficient, in and of itself, to elicit these responses" (Draft *Dioxin Reassessment*, p. 9-78).

A number of statements by EPA (in its Draft *Dioxin Reassessment* and in recent revisions of that document) and by the SAB (in reviewing the Draft *Dioxin Reassessment*) support the conclusion that Ah receptor binding (or even enzyme induction) does not, in and of itself, explain how adverse responses, particularly cancer, occur in animals. These statements also demonstrate that little is known about the mechanistic events leading to TCDD-induced carcinogenicity in animals and even less is known about the mechanism by which TCDD might induce toxicity in humans.

EPA states:

In certain cases, no response occurs even when there is some receptor occupancy, a threshold phenomenon that reflects the biological "inertia" of the system (Ariens et al. 1960). In other cases a maximal response occurs well before all receptors are occupied, a phenomenon which reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume

that the binding response relationship for one, simple effect (such as enzyme induction) will necessarily be identical to the binding response relationship for a different effect (such as cancer). (Draft *Dioxin Reassessment*, p. 2-5).

If the binding response relationships for enzyme induction and cancer are different, it cannot be concluded with any confidence that the mechanism of action by which TCDD-induced carcinogenicity occurs is adequately understood.

Even assuming that the mechanism of carcinogenic action of TCDD in animals involves the Ah receptor, the lack of knowledge concerning the similarities and differences between human and animal Ah receptors undermines any speculation that the mechanism for the carcinogenicity of TCDD in animals applies to humans. According to EPA, there is great uncertainty on this critical point. EPA states that "[t]he human receptor has not been studied extensively, and it is unknown if the properties of the human protein differ substantially from those of the Ah receptor in animals" (Draft *Dioxin Reassessment*, p. 2-8).

Further, EPA has concluded that "there is no clear mechanistic link between CYP1A1 induction and cancer" (Draft *Dioxin Reassessment*, p. 6-27). Because many chemicals, in addition to TCDD, can induce enzymes, the role of this event in explaining carcinogenicity is unknown (particularly for non-genotoxic chemicals like TCDD).

*SAB's Review of the Draft Dioxin Reassessment Demonstrates Little is Known About the Mechanism of Action by Which TCDD Causes Cancer in Animals*

The SAB's review of the Draft *Dioxin Reassessment* (SAB review) also supports the conclusion that the mechanism of TCDD-induced carcinogenicity is unknown or, at best, is poorly understood. The following excerpts from the SAB review clearly illustrate this point:

- "[M]echanisms of action for the toxic events beyond receptor binding are largely unknown" (p. 49).
- "Much of what is purported to link the Ah receptor to specific toxic events is merely the demonstration of an association between the binding of TCDD to the receptor and an eventual appearance of an adverse effect some time later in some species.... But the possible downstream events, if they exist, between Ah receptor binding and the final toxic manifestation are not well established. Mechanism of action should mean that at least some of the intermediate steps, after Ah receptor binding and leading to the pathologic processes involved, are known to some extent.... In actual fact, the only mechanism of action involving the Ah receptor that has been worked out sufficiently well to be called the biological sequence

that describes a "mechanism of action" is the induction of cytochrome P450.... The rest of the biological consequences of TCDD exposure are yet to be described adequately and sequentially in mechanistic terms" (p. 49).

- "[T]here is a large intellectual chasm between the elegant science describing the details of the TCDD receptor and its mechanism of initiating a cellular response, and the poorly understood manifestations of the toxic events associated with an alteration of the homeostasis of an animal. The linkage between Ah receptor action and specific cellular toxicity remains undefined" (p. 50).
- "Most of the "mechanistic data" support the involvement of the Ah receptor, but say little (in the context of toxicity), about how the activation of this protein alters normal physiologic function and/or development. Risk assessments based solely on Ah receptor activation or on the existing knowledge of CYP1A1 induction are unlikely to provide a biologically defensible prediction (quantitatively or qualitatively) of likely toxic outcomes in humans, particularly under low exposure scenarios" (p. 49).
- "[H]ow convincing is the evidence for the purported mechanisms that link receptor binding to toxic effects in humans? Unfortunately, the evidence is quite mixed" (p. 51).

#### EPA's Recent Revisions to the Draft Dioxin Reassessment

EPA's recently revised Chapter 8 of the *Dioxin Reassessment* (Dose-Response Modeling, January 27, 1997) and its partially revised risk characterization for the *Dioxin Reassessment* (Draft *Integrated Risk Characterization for TCDD*, Sept. 24, 1996) continue to demonstrate that the mechanism of action by which TCDD might induce tumors in laboratory animals is still unknown.

EPA expresses considerable uncertainty concerning the significance of Ah receptor binding and carcinogenicity. For example, EPA acknowledges that "[t]he relationship between Ah receptor binding and carcinogenicity of TCDD is less clear" (Revised Chap. 8, p. 6). EPA also states that, "[t]he induction of CYP1A proteins are perhaps the best characterized responses to dioxins. The relevance of these proteins to the toxic effects of TCDD are controversial" (Revised Chap. 8, p. 91). EPA also states:

- "Most of the mechanistic or dose-response information on dioxin's effects has been generated on changes in gene expression of single genes such as CYP1A1 induction. There is only limited information on the complete interaction of biochemical, molecular, and biological events that are necessary to produce a frank toxic effect such as cancer..." (p. 129).
- "The development and implementation of a complete mechanistic [risk assessment] model for

the effects of TCDD identified several areas where future research is needed. Of critical utility would be data and models which are able to directly link gene expression with toxicity in a mechanistic fashion" (p. 141).

In its Draft *Integrated Risk Characterization for TCDD*, EPA further illustrates how poorly the mechanism of action by which TCDD might induce tumors in laboratory animals is understood. EPA states:

- "The fact that TCDD may induce a cascade of biochemical changes in the intact animal raises the possibility that dioxin might produce a response such as cancer by mechanisms that differ among tissues.... One possible mechanism discussed is that TCDD might activate a gene(s) that is directly involved in tissue proliferation. A second mechanism involves TCDD-induced changes in hormone metabolism, which may lead to tissue proliferation ...which might lead to indirect mutagenic effects. Thus, while this reassessment as identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species" (p. 72, emphasis added).
- "The ability of TCDD...to modulate a number of biochemical parameters in a species-, tissue-, and temporal specific manner is well recognized. Despite the ever expanding list of these responses over the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of these changes and the degree to which they are related to the biological and toxic endpoints elicited by these chemicals" (pp. 74-5).
- "Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity and in which species" (p. 72).
- "Thus, the mechanisms by which many, if not most, of the biochemical processes are altered by TCDD treatment remain to be determined. Nevertheless, it is presumed based on the cumulative evidence available...that all of these processes are mediated by the binding of TCDD to the Ah receptor" (p. 75).

Based upon these statements, it is simply not possible to support an argument that the mechanism by which TCDD induces cancer in laboratory animals (much less in humans) is known or understood.

*IARC's Recent Dioxin Review Demonstrates that Little is Known About TCDD's Mechanism of Carcinogenicity*

IARC's TCDD Monograph (Appendix A to the RC Document) demonstrates that the mechanism by which TCDD induces cancer in laboratory animals is not well understood. In discussing mechanisms of carcinogenicity, IARC first admits that the precise role of Ah receptor activation in TCDD carcinogenicity "remains unclear" (IARC Monograph, p. 331). IARC then speculates concerning potential mechanisms involved in TCDD carcinogenicity. It suggests that effects on gene expression, oxidative damage, cell transformation, cell proliferation and tumor promotion, or suppression of immune surveillance could play a role. IARC, however, provides little or no support for these proposed mechanisms. For example, concerning gene expression, IARC only notes the induction of CYP1A1 and CYP1A2 and concludes that "The role, if any, of the induction of these [related] genes in carcinogenesis by TCDD is unclear." Concerning oxidative damage, IARC notes that the "relevance of these high dose studies [demonstrating oxidative damage] is questionable" (IARC Monograph, p. 332). Regarding cell transformation, IARC admits "conflicting results" (IARC Monograph, p. 340). Finally, IARC admits that the results of studies concerning the effect of TCDD on immune function in humans are inconsistent. Therefore, for each of IARC's hypotheses concerning possible mechanisms of TCDD carcinogenicity, the data are inconclusive, inconsistent, or of questionable relevance to humans.

**Conclusion**

Based on the totality of the available epidemiology and mechanism of action data, the most that can be concluded is that TCDD should be listed as *Reasonably Anticipated to be a Human Carcinogen*. Clearly, the available data do not support NTP's proposal to change its current listing of TCDD to *Known to Be a Human Carcinogen*.

Unlike substances on NTP's current list of known human carcinogens, the epidemiological weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Despite a great deal of study on numerous populations exposed to varying amounts of TCDD (some of them having extremely high exposure), the epidemiological data do not permit a conclusion that TCDD is a known human carcinogen. Indeed, three independent reviews (EPA, SAB and IARC) have concluded that the totality of the epidemiological data on TCDD

carcinogenicity are only "limited" or "suggestive." Such data do not provide "sufficient evidence" to allow NTP to classify TCDD as *Known to be a Human Carcinogen*.

Based upon numerous statements from the Draft *Dioxin Reassessment*, the SAB's comments on the Draft *Dioxin Reassessment*, and on EPA's revised versions of Chapter 8 on Dose-Response Modeling as well as the Draft *Integrated Risk Characterization for TCDD*, the mechanism of action by which TCDD induces tumors in laboratory animals is unknown or, at best, uncertain. While it is universally accepted that TCDD binds to the Ah receptor, beyond this initial event, the sequence of events leading to tumor development is essentially unknown. In fact, there are no peer reviewed publications which articulate the mechanism of action by which TCDD induces tumors in animals.

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